

Proof of Concept¹

iGEM 2022, University of Sheffield

March 25, 2023

¹ from <https://2022.igem.wiki/sheffield/proof-of-concept>

Showing off our functional hardware and software

Controlling Temperature of the Toroidal Bioreactor

We ran some heating tests on our bioreactor to determine the rate of heating, as well as how steady the temperature remains with the housing. After three attempts we generated the following results:

Attempt	Temp1, °C	Time1	Temp2, °C	Time2	Rate, °C/min
1	23.9	09:07	30.4	9:24	0.38
2	27.4	10:17	28.7	10:22	0.26
3	24.0	10:20	37.1	11:00	0.32

All attempts started with an initial ambient temperature of 18°C. Attempt 1 was done to determine the time it would take to heat from ambient to 30°C, which was the optimal temperature for overnight cultures of *V. natriegens*. Attempt 2 was performed to determine if we were able to maintain a constant given temperature, and was a success. Attempt 3 was to investigate how long the bioreactor took to heat from room temperature 37°C, which is *V. natriegens* optimal growth temperature.

All of our attempts were a success. We achieved optimal overnight culture temperature, maintained a constant given temperature, and then proved that the bioreactor is capable of achieving the ideal growth temperature for our chassis organism.

Recording Data of the Bioreactor

Our bioreactor is capable of recording data to the internal flash of the Raspberry Pi Pico. The following code works with the standard Python file I/O code:

```
with open("test.txt", "a") as f:
    f.write("Sup dawg!")
```

However, we needed to determine just how much data can be stored, and for how long. As the chip has 2MB of flash built in, we wrote a test program that generates a fake CSV file in order to determine how many lines of code we could store on it. The first issue we identified was that, due to the space consumed by MicroPython

there is closer to 1.4MB of available storage. We completely wiped the device of user files and determined there are 350 blocks of 4096 bytes free for 1433.6 KB of space.

One month of simulated data, taking the form 480,0.234,37.1... etc., takes 802.3 KB. This is equivalent to 43200 readings, taking one reading every 60 seconds. Hence this means it's possible to record 52 days of data at this rate, or the equivalent of approximately 75,000 lines of data.

Arguably, taking a reading every minute is potentially overdoing it when culturing in the bioreactor. Taking a reading every 2 minutes could record 104 days of data, and so on. This is more than sufficient for the demands placed upon our bioreactor, meaning that (assuming power supply remains constant the entire time) we can culture and record for potentially months on end. However, it is probably not advisable to run a culture in the bioreactor for this long as chances of contamination increase the longer it runs.

OD System of the Bioreactor

The OD system of our bioreactor functions at 800 nm wavelength. The code for the OD system is found here² As per our video we calibrated the OD system using blank media e2YT (and consequently got readings of approximately 0), and then recording an OD value of approximately 2.0 when placing a culture of *V. natriegens* in the beam. This evidence shows that our OD system is fit to detect changes in turbidity, which in turn means it is suitable for use in a turbidostat bioreactor.³

² <https://gitlab.igem.org/theLostLambda/revolver/-/blob/main/Bioreactor/Code/drivers.py>

³ <https://video.igem.org/w/9DDwkLMxwvB2T12gRgLM26>

Pumping and Outlet Systems of the Bioreactor

The peristaltic pump of our bioreactor performed precisely as intended. We performed a test using bright blue dye (to aid visualisation), pumping it from an external reservoir of diluted dye and into our bioreactor. The mixing system was also functional, and dispersed the dye amongst the media (water) rapidly.

Our outlet system also performed as expected. Once volume reached a certain volume, all excess volume flowed down the outlet tube and into the waste media container. This is evidenced by the blue droplets delivered by the peristaltic pump.⁴

⁴ <https://video.igem.org/w/hJFRT8ikvxzjNdw5tuv8jv>

Mixing System of the Bioreactor

To demonstrate the efficacy of our mixing system, we added a few droplets of undiluted dye into water in our bioreactor. The water

was intentionally left undisturbed for a length of time to minimise any eddies or currents dispersing dye before the mixing system was activated. Once the dye was added, we took a high frame rate video of the system in action and then slowed it down. The video shows two things: one, that the proof of concept mixing system does effectively reach all areas of the cross-section of the toroid, and two, that reversing the mixing direction actually mixes the media much more effectively than unidirectional mixing. This is very evident, as dye is obviously concentrated in one area of the bioreactor until the mixing system reverses.⁵

⁵ <https://video.igem.org/w/dcLgn31qEq5aNkACaJy2Ko>

Successful Media Optimisation via DoE Method

We have successfully optimised *Vibrio natriegens* growth media within a design space of 9 ingredients. We achieved a final doubling time of 15.6 minutes. This is not the fastest doubling time that *V. natriegens* is capable of, but it is the fastest we have seen from media made using our set of ingredients and almost perfectly matches the rate predicted by our model. This shows that our optimisation procedure works, and we will be able to optimise our media further by including more or different ingredients in the future. For more detail, see our Media Optimisation⁶ page.

⁶ <https://2022.igem.wiki/sheffield/media-circus>

Bioinformatics Toolbox

Our bioinformatics toolbox, though still a work in progress, is already usable and useful! For more information and to check out its sleek interface, please see the software page.⁷

⁷ <https://2022.igem.wiki/sheffield/software>